[Reprinted from FOOD TECHNOLOGY, 1951, Vol. V, No. 4, Pages 145-147]

Detection of Deterioration Products of Autoxidizing Milk Fat by Infrared Spectrophotometry[a,b]

A. S. HENICK
Quartermaster Food and Container Institute for the Armed Forces, Chicago, Illinois

(Received for publication, May 29, 1950)

The course of autoxidation of milk fat was studied by changes in the infrared absorption spectra of the volatile components, in the acceptability of the fat, and in its peroxide value. Spectral changes were detectable before a trained panel of observers could detect flavor changes. Flavor changes were marked before peroxide value changed appreciably. Loss of flavor is correlated with a specific absorption band, and growth of off-flavors with several others. The nature of the deterioration products formed is discussed.

In the course of a study of the deterioration during storage of dried milk products, it had been observed that taste panels composed of trained observers frequently rejected as unacceptable products which had none of the usual chemical criteria of deterioration. Since a part of the reason for rejection was described as off-flavor believed to be due to lipid deterioration, a more thorough investigation of the autoxidation of milk fat was indicated. Storage studies of commercial dry milk fat also demonstrated that rejection due to off-flavor and odor occurs before such chemical criteria as peroxide value indicate deterioration.

Infrared examination of autoxidizing milk fat suggested itself as a possible means for detecting products of deterioration before they had accumulated in quantities sufficient for chemical estimation. Preliminary investigations indicated that the quantities of the components necessary to produce considerable off-flavor and odor are too small to be readily detectable, even in the infrared absorption spectrum, when still dissolved in the milk fat. However, sharp absorption peaks due to hydroperoxide have been observed at 2.9 μ in samples with relatively low peroxide value (PV = 2), but with distinctly off-odor. A similar peak in autoxidizing linseed oil has been reported by Daubert *(3)*.

Sharp changes in the carbonyl absorption region (6 μ) are not discernible in the whole fat, because of the prominence of the ester carbonyl absorption at 5.7 μ. The presence of carbonyl compounds was suspected, however, as they have been detected in other autoxidizing fats *(2, 5)*. It was further suspected that these carbonyl compounds should be volatile under proper conditions, since steam deodorization is an established technique for other malodorous fats; e.g., Daubert recovered hexenals from the distillate of reverted soybean oil *(2)*. Accordingly, a sample of malodorous milk fat was distilled with steam *in vacuo*, and the distillate examined by infrared absorption spectrometry. Carbon disulfide proved to be a suitable solvent for the extraction of the organic distillate. The residue from the extract was examined in the infrared, and several bands were found in the 6-μ region, especially at 5.77, 5.81, 5.91, and 6.11. These bands constituted evidence of the presence of at least one unsaturated, conjugated ketone (or aldehyde) and another which was not conjugated. Aldehyde appears to be eliminated because the distillate did not give a positive test with Schiff's reagent.

An experiment was devised to follow the development of these new substances in milk fat and to correlate them, if possible, with the taste acceptance of a milk in which the fat is recombined. A batch of fresh fat was divided, and the control portion stored at —18° C. in sealed jars. The experimental aliquots were stored in open beakers in an air oven at 45° C. Test samples were removed semi-weekly from both storage and control. In addition to peroxide value and infrared study, milks of 3.5 percent fat were made up by recombining, using a method similar to that of Krukovsky *(4)*, the storage and control fats with a standard non-fat milk solids and distilled water. The resulting milk was submitted to sensory difference testing.

Peroxide value remained essentially constant during the first 3 weeks of the test (Figure 1), and then rose, gradually at first, and after 4 weeks more sharply. Sensory discrimination data, by duo-trio test *(6)*, show a somewhat increasing awareness of deterioration during the first two weeks, which is without statistical significance. Between the 16th and 21st day the deterioration, as indicated by taste, became statistically significant, and by the 28th day had reached the level where further testing was not necessary. This is shown as the Critical Ratio (CR) in Figure 1. Subjective descriptions of the fat were changed from "buttery" to "flat" during the 1st week, then to "strong" and finally to "rancid" during the 3rd week.

During this 28-day period, the infrared spectra of the whole stored fats showed no discernible changes. But the spectra of the steam-volatile components were most interesting. Only the 3-μ and the 6-μ regions have been examined in detail, since it is in these regions that most significant changes are to be expected.

Sampling for these spectra was as follows: The distillate from steam-deodorization at 95° C. of the stored fat was condensed in a dry-ice trap. Deodorization of a 400-g. sample required 3 hours and consumed 300 ml. water. After thawing, the distillate was extracted twice with 50-ml. portions of carbon disulfide, the extract dried, and its volume reduced to about 1 ml. by evaporation. This residue of the extract was then plated dropwise on the sample cell window, the solvent evapo-

[a] Presented before the Tenth Annual Meeting of the I. F. T., Chicago, Illinois, May 22, 1950.

[b] This paper reports research undertaken at the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned number 307 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

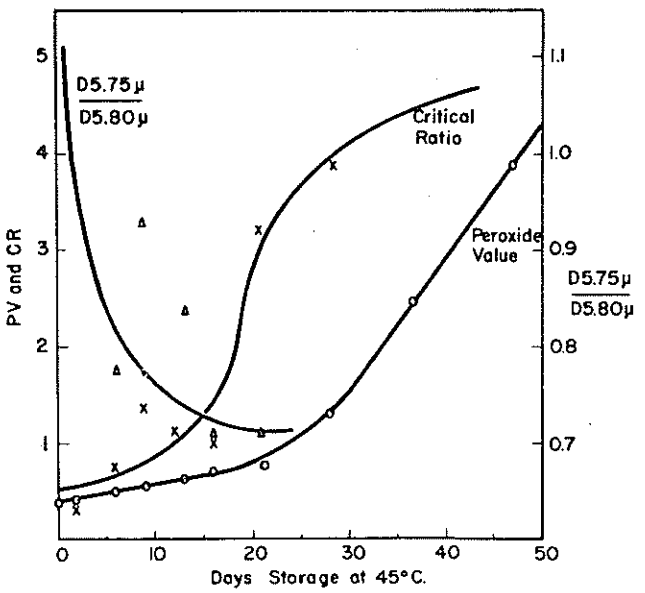

FIG. 1. Peroxide value (PV), sensory difference data (CR), and infrared criteria for milk fat stored in air at 45° C.

rated, and spectra run on a Perkin-Elmer 12-C infrared spectrometer. Since sample thickness in this method is unknown, comparison between samples is extremely difficult. The following arbitrary expedient was adopted to avoid much of this difficulty. Blanks were run on the same chart as the sample with a thick salt window in the path. Optical densities (OD) were determined by point-to-point subtraction between blank and sample on the Perkin-Elmer logarithmic paper. The peak of the greatest density for each region of interest was then set arbitrarily at OD = 1, and the densities at all other points factored appropriately. The resulting factored densities were then plotted on single-cycle semi-logarithmic paper so that samples could be compared visually. Hydroxyl absorption at 3.1 μ increases in intensity and shifts slightly to a longer wave length, indicating increasing association.

The changes in absorption in the 6-μ region of these samples are shown in Figure 2. In the distillate from the fresh fat only two peaks are observed, one at 5.80 μ, ascribed to diacetyl by Rasmussen *(7)*, and one at 5.75 μ which has not yet been assigned. Upon storage, the peak at 5.80 μ remains quite constant, while that at 5.75 either disappears or shifts to 5.70. At 45° C. this occurs within the first 6 days, while at —18° C. it is only partially complete after 4 months. It is during this period that the flavor changes from "buttery" to "flat." It is upon this evidence that the presence of a sharp peak at 5.75 μ can be used as indication of freshness in the fat, and one at 5.70 μ as an indication of flavor loss.

Upon longer storage, new peaks appear at 5.84 and at 5.91 μ. The former is probably due to a simple ketone *(7)*. The latter and a smaller peak at about 6.10 μ become increasingly strong with time, and are ascribed to a conjugated enone or enal. These bands are shifted about 0.1 μ each from those expected for isolated C = O and C = C respectively *(7)* Although there is insufficient spectral evidence to permit differentiation between ketone and aldehyde, ketone is preferred because these distillates did not give the color test with Schiff's reagent. This ketone is believed to be associated with the off-flavor which develops in the fat on storage since similar substances have been reported found in autoxidizing methyl linoleate *(1, 2, 4)*. To date no mechanism for its formation has been offered.

DISCUSSION

Since spectral evidence for a ketone is present before the end of the first week of storage at 45° C., while sensory discrimination appears significant only after two weeks, it is suggested that the shape of the infrared absorption curve of the steam-vacuum distillate of milk fat in the region 5.5 to 6.20 μ is a more sensitive test of freshness than is a trained taste panel. The latter appears to be more sensitive in detecting deterioration than peroxide values. It is further suggested that a quantitative method may be developed whereby the flavor and acceptability of fats and food products containing fats can be predicted from infrared absorption measurements, and perhaps, storage life estimated. This would in large measure reduce the time required for determination of acceptance, and eliminate much of the element of guess work still present in panel testing.

SUMMARY

Infrared absorption studies of the steam volatile components of autoxidizing milk fat have indicated that this

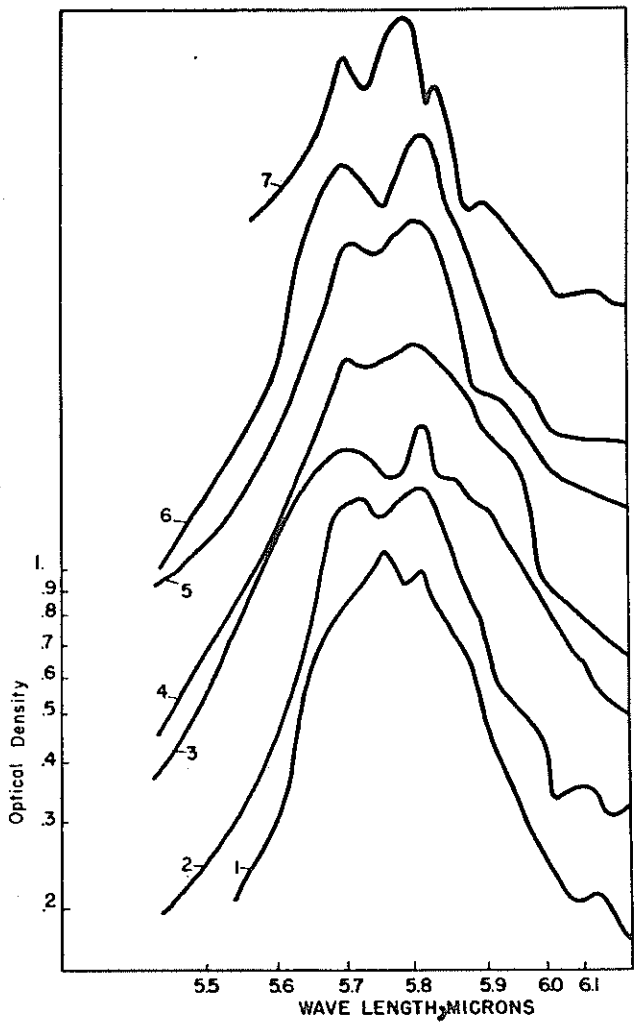

FIG. 2. Infrared absorption spectra in the carbonyl region of distillates from milk fat (each line vertically displaced 0.2 O. D. units). 1. Fresh. 2. Stored 4 months at —18° C. 3-7. Stored at 45° C. 6, 9, 13, 16, and 21 days, respectively.

method can detect changes before they are apparent by chemical or sensory means. The absorption maxima of these components show that they contain one or more simple ketones (or aldehydes), and a conjugated ketone.

ACKNOWLEDGMENT

The cooperation of Mr. D. Peryam and the Food Acceptance Branch of this Institute in conducting and evaluating the duo-trio test for acceptability is gratefully acknowledged.

LITERATURE CITED

1. Bergström, S. The oxidation of linoleic acid with lipoxidase. *Archiv. Kemi, Mineral Geol.*, **21A**, No. 15, 1 (1945).
2. Dugan, L. R., Jr., Henick, A. S., and Beadle, B. W. An infrared absorption study of autoxidized methyl linoleate. *J. Am. Oil Chem. Soc.*, 26, 681 (1949).
3. Honn, F. J., Bezman, I. I., and Daubert, B. F. Infrared absorption of hydroxy compounds in autoxidizing linseed oil. *J. Am. Chem. Soc.*, **71**, 812 (1949).
4. Krukovsky, V. N., Guthrie, E. S., and Whiting, F. Lipid deterioration in dairy products. The stability of milk fat and fat-soluble vitamins as determined by the re-emulsification test. *J. Dairy Sci.*, 31, 961 (1948).
5. Lundberg, W. O., and Chipault, J. R. The oxidation of methyl linoleate at various temperatures. *J. Am. Chem. Soc.*, 69, 833 (1947).
6. Martin, C. J., Schepartz, A. I., and Daubert, B. F. Flavor reversion in soybean oil. IV. Isolation of reversion compounds in soybean oil. *J. Am. Oil Chem. Soc.*, 25, 113 (1948).
7. Peryam, D. R., Swartz, V. W. Measurement of sensory differences. *Food Technol.*, 4, 390 (1950).
8. Rasmussen, R. S., Tunnicliff, D. D., and Brattain, R. R. Infrared and ultraviolet spectroscopic studies on ketones. *J. Am. Chem. Soc.*, 71, 1067 (1949).